ZOOLOGIA 37: e54982
ISSN 1984-4689 (online)
zoologia.pensoft.net

RESEARCH ARTICLE

The recapture of *Leptopanchax opalescens* (Aplocheiloidei: Rivulidae), a critically endangered seasonal killifish: habitat and aspects of population structure

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http://zoobank.org/A8927320-E2B6-4B77-A6D2-D37E1D131CCA

ABSTRACT. *Leptopanchax opalescens* (Myers, 1942) is a small seasonal killifish endemic to coastal drainages in the state of Rio de Janeiro, Brazil. *Leptopanchax opalescens* was recaptured in temporary open vegetation pools (22°42.35'S, 43°41.59'W) in the hydrographic basin of the Guandu River, state of Rio de Janeiro, Brazil, after eight years without records. Information on habitat and population parameters (Capture per unit area (CPUA), size, sex ratio and length-weight relationship (LWR)) are given to provide subsidies for the conservation of this species. Specimens were captured during the rainy season (January-April 2020) in shallow pools (mean depth = 21 ± 12 s.d. cm), with relatively acidic pH (5.2 ± 0.8) and low concentrations of dissolved oxygen (3.5 ± 1 mg/L). The estimated CPUA of the population was 0.89 fish/m^2. The total length (TL) of males ranged from 21.1 to 28.8 cm, and females ranged from 17.0 to 31.2 mm. Males were significantly larger (t-test = 2.04, $p < 0.05$) than females, which were in greater proportion (2.83Female: 1Male, $p < 0.001$). A positive allometric coefficient (b = 3.19) was found through length-weight relationship curve, indicating that individuals of the species gain proportionally more increment in weight than in length. *Leptopanchax opalescens* is a critically endangered species, and the results of this study contribute to the knowledge of the distribution, population structure and conservation of the species and its natural habitats.

KEW WORDS. Annual fish, Atlantic forest, conservation, Rio de Janeiro, southeast Brazil, threatened fauna

INTRODUCTION

Rivulidae (Cyprinodontiformes) is a diversified group of killifish with about 456 valid species occurring between southern Florida and northeastern Argentina (Costa 2011, Fricke et al. 2020). Rivulids are sexually dimorphic, secondary freshwater fish, small as adults, and have unique reproductive characteristics among the vertebrates (Loureiro et al. 2018). Many rivulids have a characteristic annual or seasonal life cycle and no generation overlap (Furness 2016, Reichard 2016). Growth, maturity and reproduction occur in a single, short rainy season that favors their development. Through embryonic diapause, the eggs of these fish survive the dry season that the juveniles and the adults cannot tolerate (Furness 2016). Besides dissection, the seasonal habitats of Rivulidae also pose other challenges, including limited energy, space and time for sexual maturation and reproduction (Loureiro et al. 2016). In addition, annual killifish are adapted to habitats that are often subject to a high degree of environmental variation. These environments reach extreme parameters (e.g., high temperatures, low oxygen concentrations, acidic waters) that approach the limits of vertebrate survival (Podrabsky et al. 2016).

Leptopanchax Costa, 2016 comprise six valid species – *L. aureoguttatus* (Cruz, 1974), *L. citrinipinnis* (Costa, Lacerda & Tanizaki, 1988), *L. itanhaensis* (Costa, 2008), *L. opalescens* (Myers, 1942), *L. splendens* (Myers, 1942), and *L. sanguineus* Costa, 2019 – endemic to the Atlantic Forest Biome (Costa 2009a, Fricke et al. 2020) and distributed between the Maricá Lagunar system (22°55'S) in southeastern Brazil and the hydrographic basins draining to Paranaguá Bay (25°31'S), southern Brazil (Costa 2016). Two species, *L. citrinipinnis* and *L. opalescens*, are found in temporary pools within open vegetation formations, including coastal restingas, while the other species of *Leptopanchax* are found in shallow seasonal channels within dense rainforest

(Costa 2008, 2016, 2019, Costa et al. 2019). All species in this genus are very vulnerable, and four out of the six species are listed as Critically Endangered (CE) in the Red Book of Brazil's Endangered Fauna (ICMBio 2018). *Leptopanchax sanguineus*, although recently described (2019), is possibly "born" extinct (Costa 2019). Habitat loss and fragmentation are the main threats to rivulids (Costa 2009a). Swamps and temporary pools have been drastically destroyed, both in agricultural areas and in areas undergoing urbanization, through deforestation, drainage and landfills (Castro and Polaz 2020, Calhoun et al. 2017). The scarcity of ecological information on the life history of *Leptopanchax* species does not help their preservation.

Leptopanchax opalescens is distinguished from all other species of *Leptopanchax* by the unique coloration and pigmentation pattern of the body and fins in males (Costa 2009a, 2019). This species was initially described by Myers (1942) as *Cynolebias opalescens*, being later synonymized as *Leptolebias fluminensis* (Faria & Muller, 1937) and as *Cynolebias nanus* (Da Cruz & Peixoto, 1983). More recently, Costa (2016) described a new genus (*Leptopanchax*) when its current valid status was defined as *Leptopanchax opalescens* (Myers, 1942). Records of this species are rare and the last one occurred more than eight years ago (Costa 2013). Little information is available on the life history, ecology, and population parameters of most species in the group. In this study, we assessed some population parameters of *L. opalescens* to support conservation efforts. New occurrence locations are presented, and information on the ecology (habitat description) and population parameters (Capture per unit area-CPUA, individual size, sex ratio and length-weight relationship) are provided for the first time for the species.

MATERIAL AND METHODS

The study area comprises temporary pools located approximately 4.6 km from the middle reaches of the Guandu River (hydrographic basin of the Guandu River), which drains into the Sepetíba Bay, state of Rio de Janeiro, Brazil. The locality of occurrence is an industrial site of BRF S.A (22°42.35'S, 43°41.59'W) in the municipality of Seropédica. The sampling area is part of the Atlantic Forest Biome, but a large part of this original cover has already been lost, and shrubs and grasses currently predominate. The climate is seasonal tropical, with rainy summers and dry winters (Aw climate, according to the Köppen – Geiger classification). The annual average temperature is 23.9 °C, with averages of 26.6 °C in summer and 21.4 °C in winter. The average annual rainfall is 1,354 mm, with rainy season between October and April.

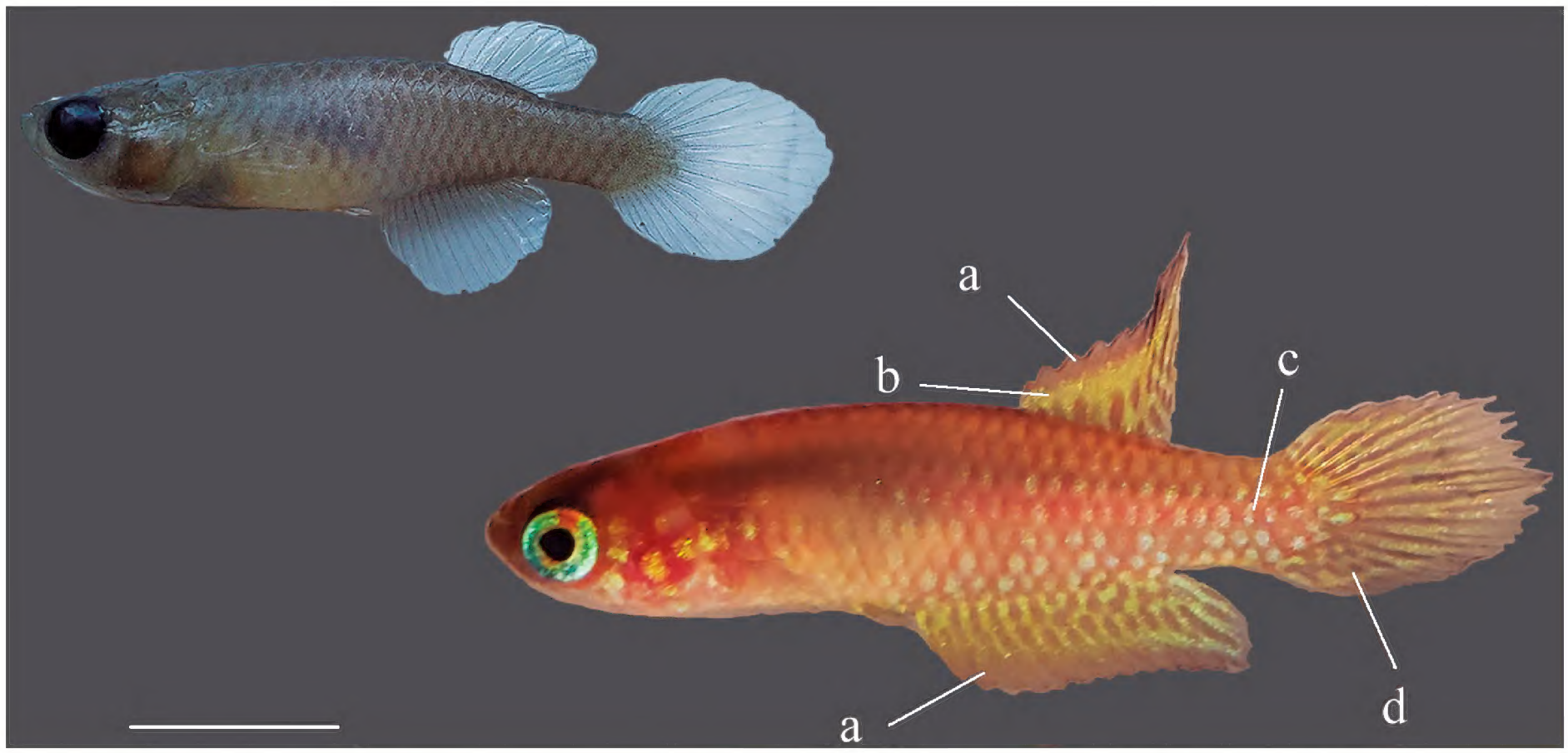
Monthly fish sampling and measurements of environmental variables were carried out simultaneously between November 2019 and April 2020. Sixteen (16) sites were delimited, each 1 m^2, where the collectors searched for fish for two minutes, totaling 96 samples (16 sites × 6 months). The sampling sites covered different types of wet habitats, from the edges to the bottom of the temporary pools. Fish were collected with immersion nets (hand net with an oval shape, 50 x 40 cm, 1 mm of panel mesh size). After capture, they were anesthetized with hydrochloride benzocaine (50 mg/l) and euthanized in situ. In the laboratory, the fish were measured (precision 0.01 cm), weighed (precision 0.001 g), fixed in 10% formalin and, after 48 hours, preserved in 70% ethanol. In order to reduce the impacts of sampling on fish populations, many live specimens were returned to the pools. The fish were identified and sexed according to Costa (2009a, 2016, 2019). The material was collected under the permission of IBAMA/ICMBio (authorization #10707) and vouchers were deposited at the Ichthyological Collection of the Fish Ecology Laboratory of the Universidade Federal Rural do Rio de Janeiro (LEP-UFRRJ # 2576-2578) and in the Ichthyological Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ # 51962).

The water physicochemical variables of temperature (°C), dissolved oxygen (mg/L), redox potential (mV), pH, electrical conductivity (mS/cm) and turbidity (NTU) were measured using a multiprobe model Horiba U-52 G (Shanghai, China). Depth (cm) was measured using millimeter rulers and a digital probe (SpeedTech SM-5).

Abundance was assessed by the total number of fish caught and expressed as a catch per unit area (CPUA: number of individuals/m^2). A Student t-test was performed to compare the differences between the mean of the total body length (TL) between males and females. A possible bias in the population sex ratio was assessed by comparing the expected rate of 1: 1, and tested with a chi-square test (χ^2), with a 95% of significance level. The length-weight ($W = a \times TL^b$) relationships (LWR) based on measurements of 39 individuals (males + females) was estimated by linear regression on the transformed equation: log (W) = log (a) + b log (TL) (Le-Cren 1951), where W is body weight (g), TL is total length (cm), a is the y-intercept and b is the slope (Froese 2006). The areas of occupancy (AOO) for the population were calculated using satellite images in Google EarthPro (version 7.3). The boundaries of the temporary pools were determined according to Lanés et al. (2014), based on visual observations of the watermarks and in the vegetation indicators (e.g., plants with morphological, physiological, or reproductive adaptations to prolonged saturation/flooding).

RESULTS

A total of 86 individuals of *L. opalescens* (18 males, 51 females and 17 with undefined sex, Figs 1, 2) were captured in temporary water pools close to the middle reaches of the Guandu River basin (Figs 3, 4). Of these, about 39 live specimens (45.3% of the total captured) were returned to the pools. This is a new area in the proximity of the Guandu River with no previous record of this species. *Leptopanchax opalescens* (Figs 1, 2; Fig. S1) showed a marked sexual dimorphism: while the females have a light brown color and hyaline fins (Fig. 1), the males have a



Figures 1–2. Specimens of *Leptopanchax opalescens*: (1) female, 24.0 mm TL, hyaline bode and fins; (2) male, 28.8 mm TL). Letters indicating patterns of body and fin color in male: (a) red band on the distal margins of the dorsal and anal fins; (b) gold band below and parallel to the red band in the dorsal fin; (c) red body with diffuse light spots; (d) vermiculate red spots in the anal fin. More photos are available in the Fig. S1). Scale bar: 5 mm.

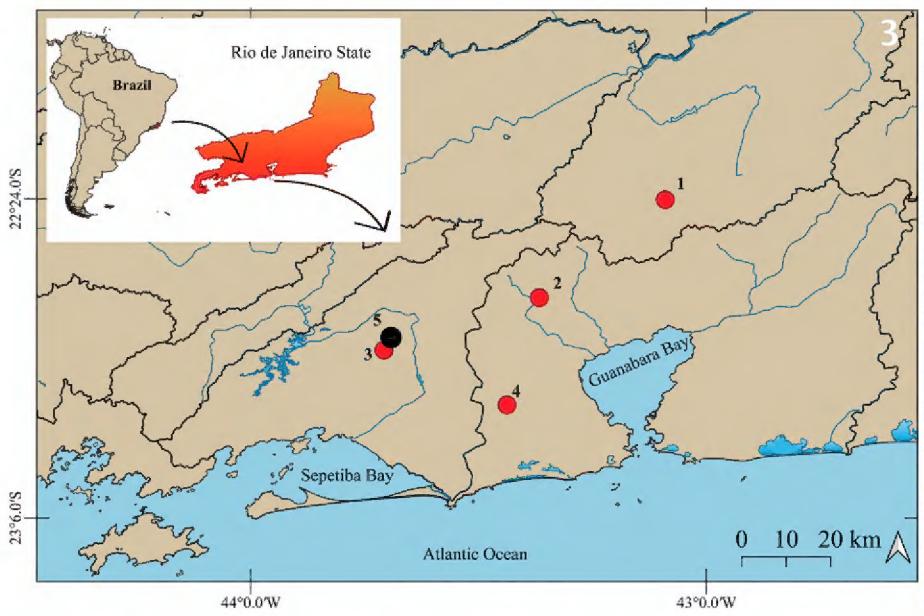
very striking color (Fig. 2), which allow the differentiation of *L. opalescens* from other similar species occurring in the Atlantic Forest biome (Costa 2019), such as: (i) dark red band well delimited on the distal margins of the dorsal and anal fins (Fig. 2a), (ii) iridescent golden band on the dorsal fins located below and parallel to the red bands (Fig. 2b), (iii) red body with diffuse light spots (Fig. 2c); (iv) caudal fin with dark red vermiculate spots (Fig. 2d). This color pattern in males corroborates those presented in the identification keys of *Leptopanchax* species (Costa 2019). In addition to the staining patterns, the meristic counts (e.g., number of fin rays, number of lateral and transversal line scales; Table 1) of the specimens overlap with the reference values presented by Costa (2009a) for *L. opalescens* and with the holotype of *Cynolebias nanus* (= *L. opalescens*, MNRJ# 10621).

The average monthly total CPUA was only 0.89 fish/m^2 from November 2019 to April 2020. The CPUA achieved the highest values between January and March 2020 and there were no records in November-December 2019 (Fig. 5). The estimated areas of occupancy (AOO) of the population found in Seropédica was defined as 8,200 m^2 (0.01 km^2). From May until the beginning of the rainy season, the pools where the rivulids were previously captured are completely dry.

Leptopanchax opalescens was recorded in a very typical habitat structure, characterized by small and shallow (<40 cm) and irregular temporary pools formed in small ground depressions (Table 2). The pools had different areas and shapes, depending on the topography of the terrain and the rainfall accumulation in the previous days; most pools were connected,

Table 1. Morphometrics (minimum-maximum) and meristic data of *Leptopanchax opalescens* (male, N = 7, female, N = 15). Measurements presented in millimeter and in relative proportion (%) of the head and body length. The proportions are mean ± standard deviation. Reference data described by Costa (2009a) and measurements of the holotype of *C. nanus* (= *L. opalescens*, MNRJ #10621) were also included.

Mophometrics	Male	Female	Costa (2009a)	*C. nanus* Holotype
Total lenght (TL, mm)	21.7–28.1	18.5–26.7		27.2
Standard length (SL, mm)	17.6–24.1	15.1–21.7		22.1
Body depth	4.2–5.5	3.2–5.4		5.6
Head length	5.4–7.3	4.8–6.6		6.7
Eye diameter	2.2–2.5	1.8–2.7		2.7
% standard length				
Body depth	24.0 ± 1.1	22.7 ± 1.86		25.3
Head length	31.2 ± 3.0	31.5 ± 2.06		30.3
% head length				
Eye diameter	35.4 ± 3.5	38.4 ± 3.1		39.5
Meristics				
Lateral line scales	26	26	26	25
Transversal line scales	7	7	7	7
Circumpeduncular scales	12	12	12	11
Dorsal fin rays	12–13	10–12	12–14	13
Pectoral fin rays	13–14	13	13–14	13
Pelvic fin rays	6	6	6	6
Anal fin rays	17–18	17–19	17–19	17
Caudal fin rays	23–25	22–25	23–24	24



Figures 3–4. (3) Map of recorded occurrences of *Leptopanchax opalescens* in the state of Rio de Janeiro, Brazil (red dots). The black dot indicates the new record in this study. References of occurrences (1, base of the Serra de Petropolis; 2, villages of Cava and Tinguá; 3, Horto Florestal Santa Cruz, in Seropédica, RJ; 4, Training camp of the Brazilian Army in Gericinó, Rio de Janeiro; and 5, a terrain of the BRF S.A. in Seropédica, Rio de Janeiro) available in the Table S1. (4) Photographic record of temporary pools flooded with marginal vegetation predominantly composed of grasses, in the municipality of Seropédica, RJ.

forming a large swamp. The vegetation cover at the margins and within the pools was predominated by grasses and, to a lesser extent, macrophytes (Fig. 4), whereas trees were absent or rare in the area. Two other fish species were captured in sympatry with the rivulids in low densities; only one specimen of the native callichthyid armored catfish *Hoplosternum littorale* (Hancock, 1828) and five individuals of the non-native three spot gourami *Trichopodus trichopterus* (Pallas, 1770). The physicochemical water variables in the temporary pools indicate acidic pH (mean ± s.d., 5.2 ± 0.8), high temperatures (26.2 ± 0.8 °C) and low oxygen concentrations (3.5 ± 1 mg/L) (Table 2).

Table 2. Habitat and water environmental characteristics in the temporary pools associated with the captures of *L. opalescens* in the Guandu River basin, state of Rio de Janeiro, Brazil. Minimum-maximum (mean ± standard deviation).

Environmental characteristics	Values
Elevation (m)	34
Areas of occupancy (m^2)	8,200
Depth (cm)	2–41 (21 ± 0.12)
Temperature (°C)	24.6–27.7 (26.2 ± 0.8)
pH	3.9–6.3 (5.2 ± 0.8)
Dissolved oxygen (mg/L)	2.1–6.3 (3.5 ± 1)
Oxide-reduction potential (mV)	141–521 (303 ± 90)
Conductivity (mS/cm)	0.055–0.110 (0.089 ± 0.014)
Turbidity (NTU)	3.9–99 (23 ± 21.6)

The chi-square test showed significant differences in sex ratio, with females outnumbering males (2.8 female: 1 male, χ^2calc = 7.95 > χ^2tab = 3.84, $p < 0.001$). The individual total length (TL) ranged from 11.2 to 31.2 mm. Size in males ranged from 21.1 to 28.8 mm (mean ± s.d., 24.8 ± 2.7 mm TL) and in females ranged from 17.0 to 31.2 mm (22.3 ± 3.0 mm TL). The mean size of males was significantly larger than females (t-test = 2.04, $p < 0.05$). The length-weight relationship (LWR) with sexes pooled was determined by the following equation fitted to a potential curve: $Wt = 0.0088 \times TL^{3.199}$ (N = 39; Fig. 6). This equation corresponds to the logarithmic form, $\ln W = -4.73 + 3.199 \times \ln L$ ($r = 0.97$).

DISCUSSION

The main finding of this study is the re-discovery of *L. opalescens* after eight years without records, and the description of its population parameters in temporary pools of open vegetation in the hydrographic basin of the Guandu River, in South-eastern Brazil. The species is listed as Critically Endangered (CE) in the latest version of the Ministry of Environment's 'Livro Vermelho da Fauna Brasileira Ameaçada de Extinção' (ICMBio 2018).

It is rare to find *L. opalescens*. The last record occurred in 2012, after 31 years without records (Costa 2013). This is not the first time that this species has been captured in Seropédica, RJ. In 1984, a single male specimen "similar" to *C. nanus* (= *L. opalescens*) was captured at the Horto Florestal Santa Cruz (currently Flona Mário Xavier), in sympathy with the critically endangered species *Notholebias minimus* (Myers, 1942) (Costa 1988). Since then, the species has not been recorded in the area. Despite the limitations posed by a record based on a single specimen in a location not represented in a reference collection, the red book considered the record of *L. opalescens* in Seropédica (ICMBio 2018) as valid. The population captured in the present study, located just 2 km away from this record in 1984, confirms the occurrence of the species for Seropédica and for the drainages of the Guandu River.

The known distribution of the species is diffuse, with several records from Baixada Fluminense beginning in the

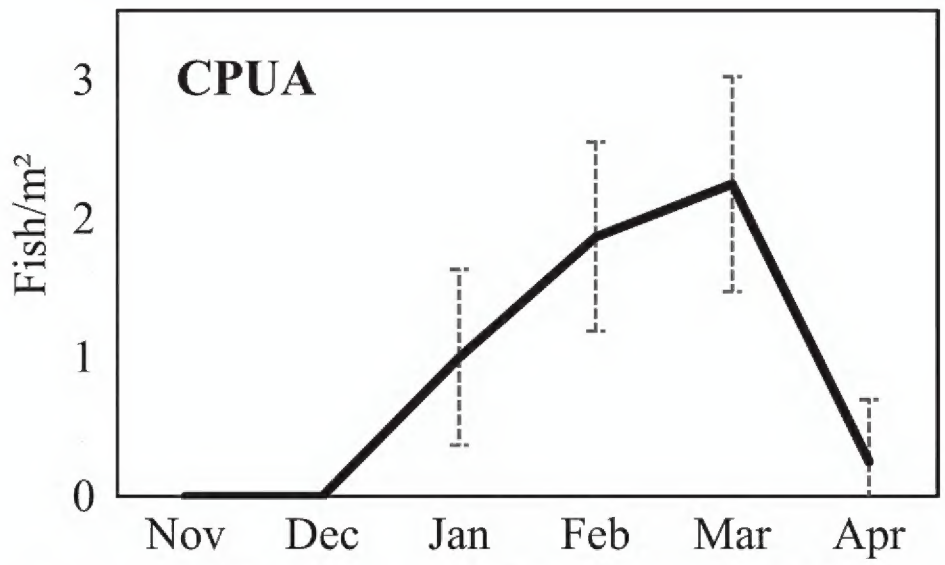


Figure 5. Monthly average and standard deviation (vertical dashes) of the catch per unit area (CPUA: number of individuals/m²) of *Leptopanchax. opalescens* between November 2019 and April 2020.

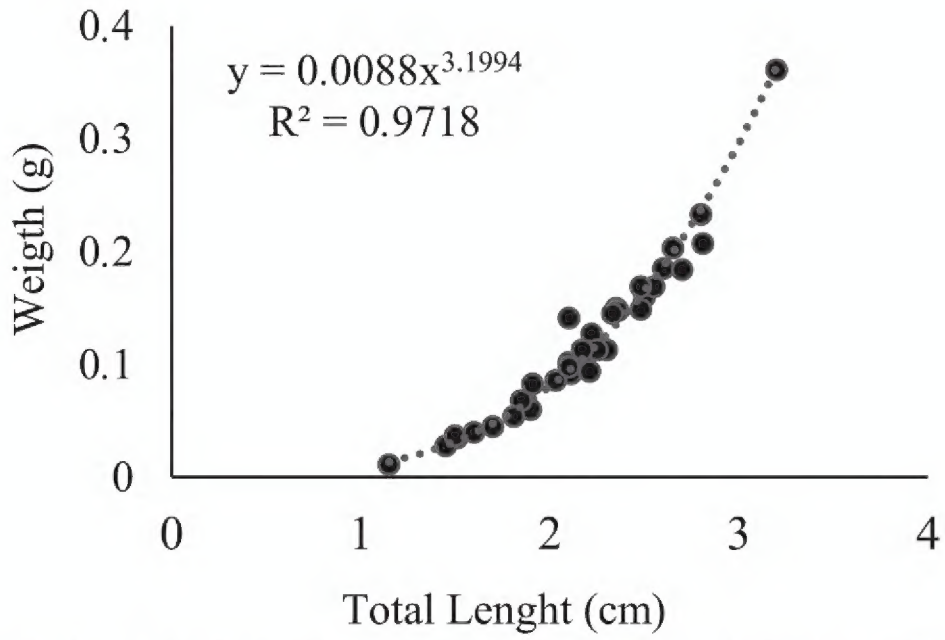


Figure 6. Length-weight relationship (LWR) of *Leptopanchax opalescens* in the Guandu River Basin, state of Rio de Janeiro, Brazil.

1940s (Souto-Santos and Beltrão 2018). Due to the urbanization and advancement of industrial and agropastoral activities, the original habitats of *L. opalescens* have been destroyed and their populations have been excised. For example, the location where *L. opalescens* was re-discovered in 2012 (22°51'06"S, 43°26'16"W) is the future site of the Rio de Janeiro's International Autodrome (Kaz 2019). During a contested environmental licensing process in the area, the species was not recaptured on location (Terra Nova 2019, Kaz 2019). In two other small hydrographic basins, Estrela and Iguaçu rivers, in the northern portion of the city of Rio de Janeiro, the species may be locally extinct (Costa 2008). The current record at the Guandu River basin is within an industrial development area. Construction there will invariably lead to losses of seasonal habitats occupied by the annual rivulids. In return, the environmental managers, upon learning about the distribution of this threatened species within the enterprise development area, remodeled the project's legal reserve area as to protect the remaining habitats of *L. opalescens*. As the annual fish usually occupy temporary pools of reduced size, this measure was a step towards the preservation of its habitat and to the conservation of this critically endangered species.

The average catch per unit area (CPUA) was only 0.89 fish/m², a low ratio that suggests that *L. opalescens* occurs at low densities. Other species of Rivulidae were also caught at values <1 fish/m², such as *Austrolebias cheradophilus* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1965) (0.86 fish/m²; Lanés et al. 2014) and *Austrolebias quirogai* Loureiro, Duarte & Zarucki, 2011 (0.33 fish/m²; Volcan et al. 2014) in southern Brazil. In general, the demography of rivulids in wetlands varies according to species, time and local conditions. It is affected by deterministic and stochastic factors, such as water availability and depth of temporary pools, connectivity of habitats, climate change, predation, competition, and reproductive, survival and mortality rates (Costa et al. 2009b, Furness 2016, Loureiro et al. 2016, Lanés et al. 2018, Garcia et al. 2019). The natural lifespan of annual fish is less than eight months, with these species disappearing before habitat desiccation (Lanés et al. 2016). Habitat condition, landscape attributes and changes in climate and hydrological variables over the hydroperiod are the factors that have the greatest influences on the spatiotemporal variation of the fish community (Volcan et al. 2019, 2020). The waters of the temporary open vegetation pools where *L. opalescens* was captured were acidic, had high temperatures and low oxygen concentrations. In fact, the annual killifish have evolved to withstand a high degree of daily and seasonal environmental variation, in terms of temperature, oxygen concentration, pH, salinity and, of course, availability of water (Podrabsky et al. 2016, Godoy et al. 2020).

Leptopanchax opalescens showed a positive allometric growth (b = 3.19), with comparatively more gain in weight than in length (Froese 2006). However, no previous references were found for the LWR of *L. opalescens* and other species of *Leptopanchax*, making it impossible to compare our results with other studies. Males of *L. opalescens* had a larger body than females, corroborating the pattern of sexual dimorphism commonly observed in other species of Rivulidae (e.g., Lanés et al. 2012). In *Austrolebias reicherti* (Loureiro & García, 2004) for example, males tend to choose larger females, which often produce larger and more numerous eggs (Passos et al. 2019). Differences in body size mediate the coexistence of annual fish in temporary pools (Volcan et al. 2019). The unbalanced sex ratio with a predominance of females of *L. opalescens* (2.8 female: 1 male), have been also reported in previous studies for other species of Rivulidae (e.g., Reichard et al. 2009, Lanés et al. 2012). Sex ratio might influence ecological conditions whenever sexual dimorphism is associated with ecological dimorphism in species with strong ecological interactions (Fryxell et al. 2015). In *Nothobranchius* killifish (Nothobranchiidae), for instance, there is a bias in the sex ratio with a higher male mortality in nature (Reichard 2016). This may be associated with males being more frequent targets of heron attacks than females (Haas 1976) and

higher male mortality results from their aggressive behavior (Reichard et al. 2014, Polačik and Podrabsky 2016). Annual fish suffer high sex-dependent size-specific mortality that is more strongly related to predation than to density-dependent processes (Reichard et al. 2018).

Currently, there are six valid species of annual fish in *Leptopanchax* and four are endemic to the state of Rio de Janeiro (Costa 2019, Fricke et al. 2020), which reinforces the importance of environmental measures and public policies in the state to preserve fish in this genus. The recovery of degraded habitats and a management plan for a wider area, together with the creation of mechanisms to facilitate the registration of legal preserves and environmental education programs are appropriate measures that can contribute to the conservation of this species. The recapture of *L. opalescens* was an important step to call the attention to this species and take actions to preserve it. The identification was based on the available literature, including comparison with the holotype of one of the species synonymized as *L. opalescens*. In view of the high degree of specialization and endemism of rivulids, osteological and genetic techniques should be used in the future to compare the known populations of *L. opalescens*.

In this study, we re-discovered *L. opalescens* in an industrial area with intense landscape modification, an indication that part of its original habitats has been already lost. After the discovery of these rivulids, the wetland areas where the population of this fish occurs were protected within the area of the enterprise. This is an example of integration between scientific results and effective environmental management practices, with demarcation of protected areas. Besides the recapture of this species, we described population parameters such as capture per unit area (CPUA), length-weight relationship (LWR), sex ratio, and size structure. These are basic information that was previously unknown. The species' occurrence data are publicly available on the Information System on Brazilian Biodiversity (SIBBr, in Portuguese) and Global Biodiversity Information Facility (GBIF) (Araújo et al. 2020). We hope that the information generated here will also reach the general public, researchers, and conservation agencies, and help the management and conservation of this annual fish that is threatened in Brazil.

ACKNOWLEDGEMENTS

We thank Paulo A. Buckup and Igor C. de Araújo Souto-Santos for receiving us at the Rio de Janeiro National Museum in these difficult pandemics times and for sharing specialized literature on this species. We thank BRF S.A. for financially supporting this project (Proc. FAPUR/BRF # CRW2523134) and allowing us to access to the study area. The fish collection was authorized by the Brazilian Environment Agency (SISBIO/IBAMA/ICMBio, Permission 560 #10707). The authors state that the research was conducted according to ethical standards. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Submitted: June 2, 2020
Accepted: October 14, 2020
Available online: December 3, 2020
Editorial responsibility: Vinicius Abilhoa

Author Contributions: GHSG: Conceptualization, investigation, writing original draft. FLKS, DLPF and WU: Investigation, formal analysis. FGA: Conceptualization, writing-original draft.
Competing Interests: The authors have declared that no competing interests exist.
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GBIF DATASET OCCURRENCE

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Supplementary material 1

Figure S1. Photographic records of *Leptopanchax opalescens* in an aquarium after three hours of capture (Photos 1–3), and record of a male specimen with more exuberant color immediately after capture in its natural habitat (Photos 4–5). Date: March 19th, 2020; Coordinates: 22°42.35'S, 43°41.59'W.

Authors: G.H.S. Guedes, F.L.K. Salgado, W. Uehara, D.L. de P. Ferreira, F.G. Araújo.

Data type: species data.

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Link: https://doi.org/10.3897/zoologia.37.e 54982.suppl1

Supplementary material 2

Table S1. References for the occurrence of *Leptopanchax opalescens* and synonyms as shown in Fig, 2.

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